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Can cooperation within the vaginal microbiome lead to the development of bacterial vaginosis?

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Despite being a controversial topic over the past few decades, *Gardnerella vaginalis* has been attributed a central role in bacterial vaginosis (BV) development. An important milestone in BV research was the discovery that the different species involved in BV were associated in a structured polymicrobial biofilm, dominated by *G. vaginalis*. Subsequent studies demonstrated that *G. vaginalis* biofilms display a high resistance to the protective mechanisms of normal vaginal microflora, as well as an increased tolerance to antibiotics. Despite the increased evidence of the pivotal role of *G. vaginalis* in BV biofilm development, the importance of the other BV-associated anaerobes should not be neglected.

There are some observational studies that correlate bacterial co-colonization between *G. vaginalis* and some other specific BV-associated bacteria during BV. Furthermore, synergistic interactions can occur between BV-associated species and *G. vaginalis*, leading to increased biofilm formation in dual-species biofilms. We hypothesized that differential bacterial interactions can occur during BV development. To test our hypothesis, we examined the ecological interactions between *G. vaginalis* and other 15 BV-associated anaerobes that we had previously shown to enhance biofilm formation by *G. vaginalis*, using a dual-species biofilm model.

Bacterial distribution and biofilm structure were evaluated by peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) method and confocal laser scanning microscopy analysis. Furthermore, the bacterial coaggregation ability was determined as well as the gene expression of virulence genes. The total biomass and the bacterial populations of dual-species biofilms were also quantified, using the crystal violet and PNA FISH methods, respectively.

Our results revealed distinct dual-species structures, between the different consortia, with at least 3 unique biofilm morphotypes. Many, but not all consortia, revealed an induction of *G. vaginalis* genes associated to cytotoxicity, biofilm formation, antimicrobial resistance and immune response.

Overall, this important research contributes to our understanding of how multi-species biofilms contribute to the development of BV. Importantly, the detected specific molecular interactions were very specific to each consortium, suggesting that not all BV- secondary anaerobes contribute to enhanced virulence.